HUNTINGTON MEDICAL RESEARCH INSTITUTES

NEUROLOGICAL RESEARCH LABORATORY

734 Fairmount Avenue

Pasadena, California 91105

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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

William F. Agnew, Ph.D.

Ted G.H. Yuen, Ph.D.

Randy R. Carter, Ph.D.

Douglas B. McCreery, Ph.D.

Leo A. Bullara, B.A.

This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program.

ABSTRACT

Work this quarter has included implantation into the cerebral cortex of 4 cats, HMRI microelectrodes having four different tip configurations (3, 6 and 12 μm diameter conical tips and one faceted tip). Of the 16 implant sites, 8 showed signs of recent or earlier hemorrhage related to the electrodes. None of the conical tip configurations protected the cortex from hemorrhage. Only the faceted electrode site showed no hemorrhage. Two of the 4 electrode sites beneath reapproximated dura showed scars associated with the electrode tracks (evidence of a previous hemorrhage) while 6 of 12 electrode sites overlaid by fibrin glue showed evidence of hemorrhage.

INTRODUCTION

This is the fifth Quarterly Progress Report in which we have investigated electrode designs and surgical techniques to obviate mechanical trauma and microhemorrhages during microelectrode insertion (QPR #'s 1, 2 and 4, 1995, and 5, 1996).

In this report, we have evaluated the following parameters: (1) Rapid electrode insertion using the stereotaxic frame-mounted axial introducer, (2) Four different tip configurations (faceted 3, 6 and 12 μm conical tips), (3) Fibrin glue overlaying vs. dural reapproximation over the operative site, and (4) Variations in implant duration (24 hours, 2 and 3 weeks).

MATERIALS AND METHODS

I. Surgical Protocol.

A craniectomy was performed over the parietal gyri (Table 1) and a horseshoe-shaped incision was made through the dura and the flap reflected to reveal the operative site. The probes were inserted with the aid of an axial introducer mounted on the stereotaxic frame (SFMAI). All electrodes were implanted into the gyrus marginalis or gyrus suprasylvius. At 12 of 16 operative sites, the dura was not resutured in an attempt to obviate mechanical damage inflicted by forces transmitted to the electrodes by traction on the dura. Rather, fibrin glue was applied over the operative site, covering the tops of the electrodes. The dura was reapproximated at the remaining four sites.

The implant duration was 24 hours for IC 139, two weeks for IC 142, and three weeks for IC 140 and 141. At the end of these periods, the animals were perfused transcardially through the ascending aorta using Karnovsky's fixative.

II. Electrodes

Four tip configurations were used in this series. The HMRI electrodes were single, 1.5 mm long epoxylite-coated Ir shafts with diameters of 50 μm and 3, 6 or 12 μm diameter conical tips. One faceted electrode was used in this series.

III. Autopsy

One day after perfusion, the calvarium and dura or fibrin glue were removed to reveal the electrodes in situ on the crown of the parietal gyrus (gyrus marginalis and/or gyrus suprasylvius). Special attention was directed towards the presence of any epi- or subdural hemorrhage or blood beneath the electrode matrices. In addition, the operative sites in the cortex were examined for a small hemorrhagic annulus encircling the electrode entry sites. The examination also included observations for inflammation or infection at the operative sites and the amount of connective tissue overgrowth covering the electrodes.

IV. Tissue Processing

Blocks of control cortex and those containing the array site were resected at autopsy and processed through ascending concentrations of ethanol and embedded in paraffin.

TABLE 1
AUTOPSY AND HISTOLOGICAL FINDINGS: HMRI ELECTRODES

IC #	TYPE ELECTR.	FIBRIN GLUE VS. DURAL REAPPROX.	DURATION OF IMPLANT	AUTOPSY	HISTOLOGY		SCAR	GLIO-SIS	NEURONS
					HEMORRHAGE	CAVITAT.			
139	12 µm	Fibrin glue	24 hours	Hemorrhage under electrode matrix	+ 65 µm dia near tip	0	0	0	N
	12 µm	Fibrin glue	24 hours	Hemorrhage under electrode matrix	0	0	0	0	+ Flat
	12 µm	Fibrin glue	24 hours	Hemorrhage under electrode matrix . Also subdural hemorrhage.	+++ 50x500 µm adjac. to track	0	0	0	+ Shrunken
	12 µm	Fibrin glue	24 hours	Hemorr. under electrode matrix	0	0	0	0	++ Shrunken
140	12 µm	Fibrin glue	3 weeks	N	0	100x250 µm	0	+++	N
	12 µm	Fibrin glue	3 weeks	N	200x600 µm adjac. to track	0	0	+++	++ Flat
	12 µm	Fibrin glue	3 weeks	N	0	0	0	++	+ Flat
	12 µm	Fibrin glue	3 weeks	N	0	0	0	++	N
141	3 µm	Dura	3 weeks	N	0	0	++ 250 at tip	+++	N
	12 µm	Dura	3 weeks	Tissue on underside of matrix	0	0	+++ 100x300 µm at tip	++	N
	3 µm	Fibrin glue	3 weeks	N	0	0	0	+	N
	12 µm	Fibrin glue	3 weeks	N	0	0	0	+	N
142	12 µm	Dura	2 weeks	N	0	0	0	+++	N
	Facet	Dura	2 weeks	N	0	0	0	+++	N
	3 µm	Fibrin glue	2 weeks	N	0	0	+ 100x150 µm at tip	+++	N
	6 µm	Fibrin glue	2 weeks	N	0	0	++ 150x200 µm at tip	++	N

All electrodes were inserted rapidly (108 cm/sec) with a Stereotaxic Frame Mounted Axial Introducer. N = Normal + = Slight ++ = Moderate +++ = Marked

Serial sections were taken from control blocks and those sectioned through the electrode tracks included any associated hemorrhages, scars, cavitations or neuronal damage. Alternate slides were stained with H & E and Nissl stains.

RESULTS

I. Autopsy.

In one animal (IC 139), hemorrhage was present under all four electrode matrices and an annular hemorrhage surrounded the electrode entry site of one of the four 12 μm tipped electrodes. Four electrode shafts were partially covered by patches of brownish tissue, presumably blood. All four of these electrodes had 12 μm diameter tips and represented all three implant durations. In a single instance (IC 141, 3 μm tip), a thin layer of unidentified tissue was present between the underside of the electrode matrix and the cortex.

II. Results - Histological Examination.

A total of 16 HMRI single-probe implant sites were studied histologically. The leptomeninges were examined for evidence of hemorrhage and infiltration by neutrophils or other cell types. Special attention was given to the state of preservation, the depth and direction of the electrodes and the appearance of all neurons, especially those near the track. The presence and extent of hemorrhages, cavitations and scars adjacent to the tracks were compared with respect to the types of probe implanted. Also noted were the appearance of neurons and the amount of gliosis near the track and the presence of inflammation.

A. 3 μm diameter conical tips.

1. IC 141, 1 implant, dural reapproximation, 3 wk. duration. A 200 x 250 μm, well organized connective tissue (CT) scar was present adjacent to the tip site (Fig. 1). Nearby neurons appeared normal. The track was accompanied by a few glial cells.

2. IC 142, 1 implant overlaid with fibrin glue, 2 week duration. A small, well organized connective tissue scar was found adjacent to the track. Normal-appearing neurons were near the track although marked gliosis accompanied the track.

3. IC 141, 1 implant, overlaid with fibrin glue, 3 weeks. There was no evidence of hemorrhage, cavitation or scars. Nearby neurons appeared normal and gliosis adjacent to the track was slight.

B. 6 µm diameter conical tip. IC 142, 1 implant, overlaid with fibrin glue, 2 weeks duration. A 150 x 200 µm, compact CT-scar was present adjacent to the tip site. Moderate gliosis closely accompanied the track and nearby neurons appeared normal.

C. 12 µm diameter conical tips.

1. IC 142, 1 implant, dural reapproximation, 2 weeks. There was no hemorrhage, cavitation or scar associated with the track. Marked gliosis surrounded the track although nearby neurons appeared normal. (Fig. 2)

2. IC 139, 4 implants, overlaid with fibrin glue, 24 hours. Because of the brief implant duration, cavitations or scars were not present. However, two implant sites showed hemorrhages in communication with the track. A small, 65 µm diameter, hemorrhage was in communication with the tip site of one electrode track while a narrow, elongated (50 x 500 µm) hemorrhage skirted the edge of another track. Gliosis had not yet developed at any track. Neurons near only one track were normal. The remaining tracks were accompanied by slightly flattened or slightly-to-moderately shrunken neurons. (Fig. 3)

3. IC 140 and 141, 6 implants, overlaid with fibrin glue, 3 weeks. Three implant sites showed no evidence of hemorrhage, past or present. Gliosis along the track was slight to moderate. At two of these sites, all neurons appeared normal while the third site showed a few mechanically flattened neurons (Fig. 4). The fourth implant site showed a fresh hemorrhage measuring 200 x 600 µm lying adjacent to the track. The cause of this hemorrhage, seen 3 weeks after electrode implantation, is unknown but probably is associated with electrode movement during daily activity. Marked gliosis was present along the track. Several neurons along the track were mechanically flattened. The fifth implant revealed the only cavitation in the entire study. The defect was 100 x 250 µm and lay adjacent to the track. It appeared that all extravasated RBC were scavenged by macrophages and hemosiderin was still present in many of the macrophages still

present within the cavitation Figs. 5A & B). Marked gliosis was present around the track although nearby neurons appeared normal.

D. Faceted tip. IC 142, one implant, dural reapproximation, two week duration. A well-organized CT sheath was present at the tip. Moderate gliosis surrounded the track. Nearby neurons appeared normal.

SUMMARY

Eight of the 16 electrode sites showed evidence of recent or past hemorrhage. The latter took the form of a cavitation in the neuropil adjacent to the track or a CT scar in apposition to the track.

When categorized by tip configuration, two of three implants having a 3 μm diameter tip showed evidence of an earlier hemorrhage. The only 6 μm diameter electrode site showed a CT scar in apposition to the tip site. Five of the 11 implant sites having 12 μm diameter tips showed a total of 3 hemorrhages, 1 cavitation and 1 scar.

DISCUSSION

None of the conical tipped electrode configurations prevented hemorrhage during insertion of the probe. Previously, the 12 μm tipped electrodes were considered sufficiently blunt so as to "nudge" blood vessels aside rather than pierce or rupture them. In the present study, this proved not to be the case, with almost half of these sites showing fresh hemorrhages or scars.

Photolithographic electrodes were not available for the present series. Inasmuch as only 3 of the 42 Michigan probes described in the last QPR were associated with hemorrhages, and since these were inserted manually, it appears advisable to conduct a series of implants using manually inserted 12 μm (and other size) tip configurations. Of the 6 electrode sites which showed flattening or shrinkage of nearby neurons, 4 of these were present at the 4 sites implanted for 24 hours. It appears that, in the main, slight mechanical disturbance of neurons was resolved by 2 or 3 weeks after surgery. However, it has been shown in our previous series that markedly flattened neurons often remain flattened even several weeks after electrode implantation. At all but the 24-hour implant sites, gliosis ranged from slight to marked and was present along the shaft and not isolated to the tip site. At 5 implant sites (IC 141, 142), marked inflammation at the surface of the cortex was presumed to be due to contamination of the stored Epoxylite monomer. New monomer has been ordered.

Fibrin glue was used to overlay 12 of the 16 implants of all types and six of the nine implant sites which sustained hemorrhages. In view of this, the downward or lateral mechanical forces caused by reapproximation of the dura cannot be solely responsible for hemorrhages along the track. However, when the present series with a 50% incidence (6/12) of bleeding at fibrin glue sites is compared with a previous series having 34 hemorrhages at 35 implant sites where the dura was reapproximated, the present results are considered significantly superior. In other series, the results comparing fibrin glue with dural reapproximation are less clear-cut and indicate the need for further studies and clarification. It is anticipated that fibrin glue will be a valuable aid in establishing positional stability of the cables for both HMRI and Michigan electrodes in upcoming experiments.

WORK NEXT QUARTER

Twelve μm (and smaller) diameter tipped electrodes arranged in a circular pattern will be inserted manually into the parietal gyri. It is anticipated that, to avoid dimpling and more readily penetrate the pia, some of the electrodes in each array will be longer than others, thus "staggering" the forces exerted on the pia.

Although it is presently inconclusive whether the use of fibrin glue is superior to dural suturing, we will employ the former method inasmuch as it appears to exert much less of a mechanical disturbance than the reapproximated dura. To further diminish mechanical forces exerted on the cortex, the diameter of the electrode shafts will be reduced to 38 μm.

We have begun to implant the electrically-active silicon microprobes supplied by the University of Michigan. Our first experiences with these will be presented in the next QPR.

Fig. 1. Animal IC 141. 3 μm diameter electrode tip; dural reapproximation; implant period 3 weeks. This and all succeeding micrographs were taken from 8 μm thick paraffin sections cut vertically through the electrode tracks. Here, an organized connective tissue scar (S) is present at the tip site of the track (T). Numerous glial cells (G) skirt both the track and the scar. At a higher magnification, nearby neurons appeared normal. Bar = 50 μm.

Fig. 2. Animal IC 142. 12 μm diameter electrode tip; dural reapproximation; implant period 2 weeks. Oblique segment of the track (T) 175 μm above the 1,625 μm deep tip site. Marked gliosis surrounds the track. Nearby neurons appear normal. Bar = 50 μm.

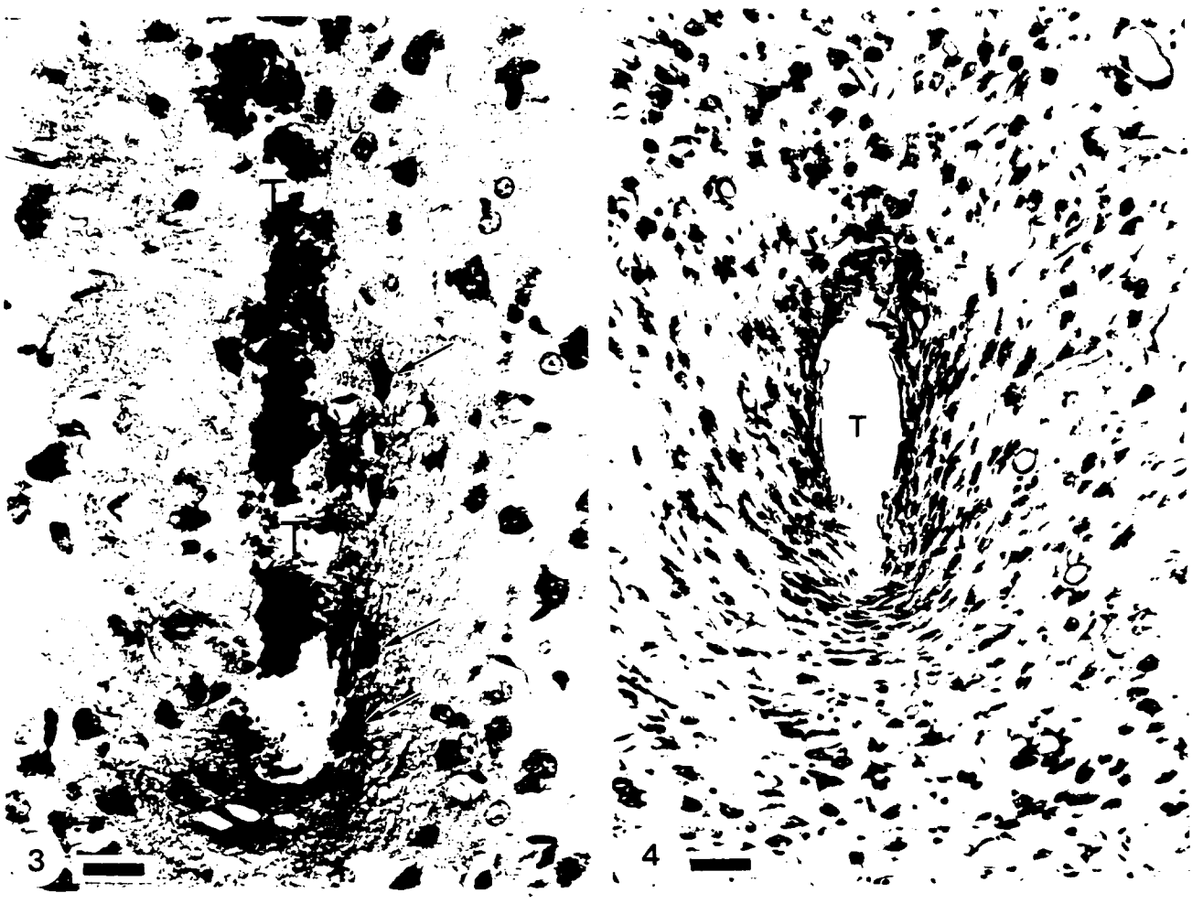

Fig. 3. Animal IC 139. 12 µm diameter tip; overlaid with fibrin glue; implant period 24 hours. Fresh hemorrhage is confined to the track (T) and probably represents seepage from a ruptured pial vessel at the point of entry. The tip site is present at a depth of 1,125 µm. Because of the short duration of implant (24 hours), the edges of the track are ragged and lack a connective tissue sheath. A few nearby neurons are shrunken and hyperchromic (arrows). Bar = 25 µm.

Fig. 4. Animal IC 140. 12 µm diameter tip; overlaid with fibrin glue; implant period 3 weeks. The electrode tip site is at a depth of 875 µm. Moderate gliosis surrounds the track (T). A few nearby neurons are mechanically flattened (not well seen at this magnification). Bar = 50 µm.

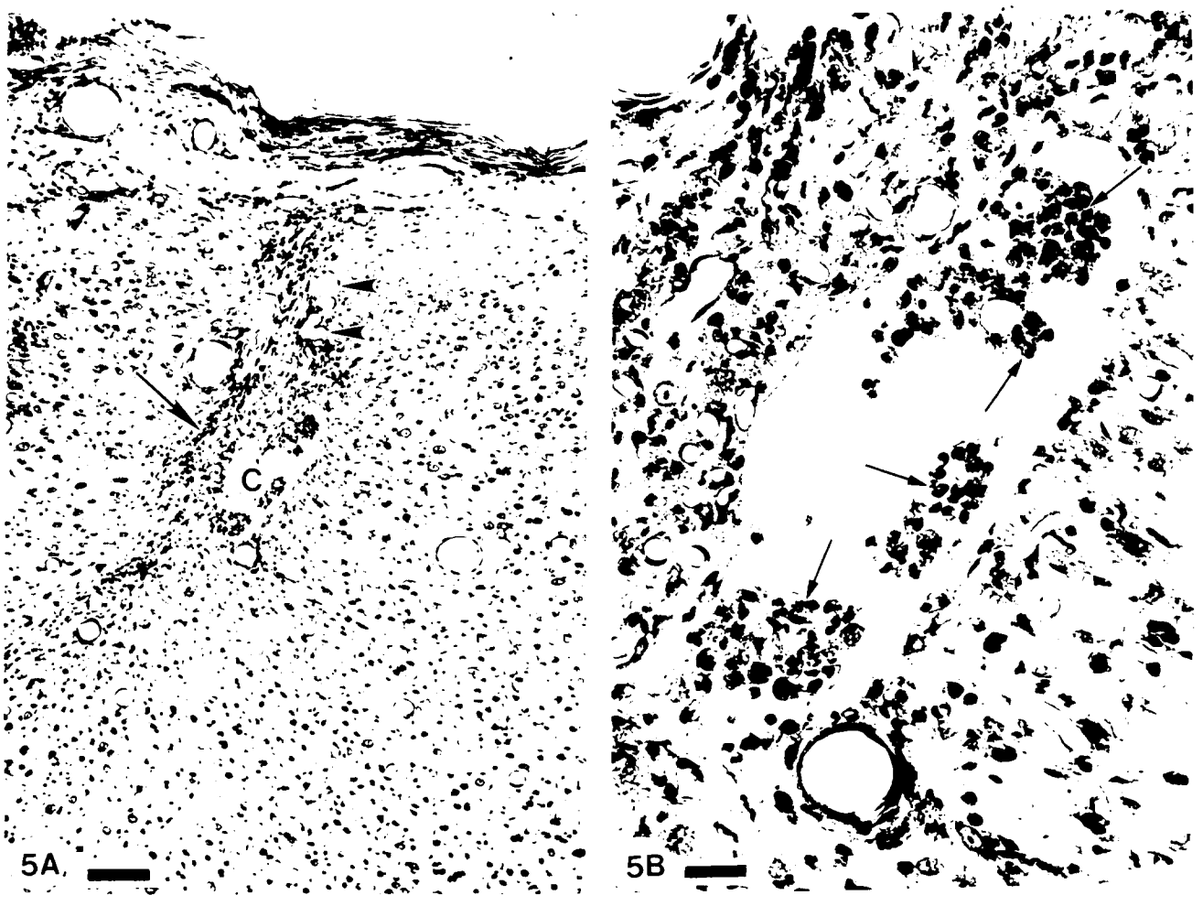

Fig. 5A & B. Animal IC 140. 12 μm diameter electrode tip; overlaid with fibrin glue; implant period 3 weeks. (A) The gliotic sheath (arrows) surrounding the track extends about 600 μm below the pia. The track is not included in the plane of section. Vascular hypertrophy (arrow heads) is present near the sheath. A large cavitation (C) lies adjacent to the sheath. Bar = 100 μm. (B) Higher magnification of cavitation in Fig. 5A. The defect is 100 x 200 μm and contains numerous hemosiderophages (arrows). At high magnification, the pink-staining hemosiderin was clearly visible within the macrophages. Bar = 25 μm.